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PROGRAM

THIRTY-SEVENTH ANNUAL MEETING
THE SOCIETY FOR INVESTIGATIVE DERMATOLOGY, INC.

Chalfonte Hotel
Atlantic City, New Jersey
April 29 to May 1, 1976

WORKSHOPS
Thursday, April 29, 1976, 7:30 P.M.

Workshop	Directors
Genetics	Lowell A. Goldsmith Durham, North Carolina
Chemical Mediators of Inflammation	Nicholas A. Soter Boston, Massachusetts Bruce V. Wintroub Boston, Massachusetts
Cell-Mediated Immunity: Analysis of In Vitro Methods and Effector–Suppressor Systems	William L. Epstein San Francisco, California Stephen I. Katz Bethesda, Maryland
Melanin Pigmentation	Walter C. Quevedo, Jr. Providence, Rhode Island Madhu A. Pathak Boston, Massachusetts Sidney N. Klaus New Haven, Connecticut
Photobiology	John A. Parrish Boston, Massachusetts
Acne	James J. Leyden Philadelphia, Pennsylvania

MORNING SESSION

THE SEVENTH IRVIN H. BLANK RESIDENT-FELLOW FORUM
Friday, April 30, 1976, 7:30–9:45 A.M.
DNA Metabolism and Its Disorders
Moderator: I. A. Bernstein
Ann Arbor, Michigan

Defective DNA Repair in Xeroderma Pigmentosum and Its Variants	James Trasko East Lansing, Michigan
DNA Repair in the Progerias	A. James Epstein Boston, Massachusetts
DNA Replication in Bloom's Syndrome	Roger Hand Montreal, Quebec
Autoimmunity to DNA	James N. Gilliam Dallas, Texas

BUSINESS AND EXECUTIVE SESSION
8:30 A.M.
John S. Strauss, Boston, Massachusetts, Presiding

SCIENTIFIC SESSION
10:00 A.M.
Richard L. Dobson, Buffalo, New York, Presiding

Presidential Address: Our Forgotten Majorities. John S. Strauss, Boston, Massachusetts.

Stephen Rothman Award to Albert M. Kligman, *Philadelphia, Pennsylvania.*

Genetic Heterogeneity in Excision of Psoralen–DNA Cross-Links in Xeroderma Pigmentosum. D. M. CARTER AND M. PAN, Department of Dermatology, Yale University, New Haven, Connecticut.

The ability to repair DNA containing photomediated cross-links of trimethyl psoralen (TMP) was studied in several types of human fibroblasts in cell culture. 200,000 cells labeled with [^{3}H]thymidine were layered onto 5–23% alkaline sucrose gradients (38.5 ml) and exposed to a deproteinizing, lysing solution (20 min, 20°C). After centrifugation (27,000 rpm, 2.5 hr, 20°C), 1-ml fractions were collected. With control cells, radioactivity due to [^{3}H]DNA was recovered in a single peak near the center of the gradient. With fibroblasts harvested immediately after nonlethal exposure to TMP (2×10^{-7} M) and UV-A (365 nm, 100 ergs/mm^2/sec, 8 min), [^{3}H]DNA sedimented faster, suggesting that photomediated TMP–DNA cross-links had been formed, and that the DNA was resistant to denaturation in alkaline sucrose.

After exposure to TMP and UV-A, some cells labeled with [^{3}H]thymidine were incubated (37°C) for 24 hr before centrifugation. With normal fibroblasts (CRL 1295), [^{3}H]DNA sedimented in the control position again, and neither caffeine (3 mM) nor hydroxyurea (2 mM) affected the return shift. With fibroblasts from patients with xeroderma

pigmentosum (XP), however, three patterns of sedimentation of DNA were observed 24 hr after exposure to TMP and UV-A: [^{3}H]DNA persisted in the fast-sedimenting position (XP CRL 1160); [^{3}H]DNA shifted to an intermediate position (XP CRL 1170); or [^{3}H]DNA returned to the control position (XP CRL 1166). These data suggest that repair of photomediated, TMP–DNA cross-links involves an initial excision step which is defective to different degrees in some, but not all, patients with xeroderma pigmentosum.

Increased Cutaneous Carcinogenic Induction with Ultraviolet Radiation and Immunosuppressive Agents. FRANK C. KORANDA, R. T. LOEFFLER, D. M. KORANDA, AND ISRAEL PENN, Division of Dermatology and Department of Surgery, University of Colorado Medical Center, Denver, Colorado.

Experiences with renal transplant patients indicate the possibility that immunosuppressive agents in conjunction with ultraviolet radiation (UVR) may have a cutaneous carcinogenic potential. For 210 days, *hr/hr* hairless albino mice were exposed to UVR and were treated with azathioprine, cyclophosphamide, and prednisone or with combinations of these agents. The UVR was administered 35 min each day, 5 days a week, using two Westinghouse FS-40 sunlamps. The immunosuppressive agents were mixed into the feed so that there were 75 mg of azathioprine per kg of feed, 50 mg of cyclophosphamide per kg of feed, 20 mg of prednisone per kg of feed, or similar concentrations with combinations of azathioprine and prednisone or cyclophosphamide and prednisone. Cutaneous malignancies occurred only in animals receiving azathioprine. Fifty-seven percent of the UVR-exposed azathioprine animals developed squamous cell carcinomas at an average time of 139 days and 56% of the UVR-exposed azathioprine–prednisone animals developed squamous cell carcinomas at an average of 156 days, compared with 18% of the control animals which developed squamous cell carcinomas at an average of 180 days. In hairless mice, azathioprine seems to have an accelerating effect on UVR carcinogenesis.

Serine Biosynthesis in Human Hair Follicles. L. A. GOLDSMITH AND T. O'BARR, Department of Medicine, Duke Medical Center, Durham, North Carolina.

Serine and its direct metabolic product glycine are the two most abundant free amino acids in hair roots and epidermis, and exceed their plasma levels manyfold. Studies to determine whether these amino acids might be directly synthesized in keratinized tissues were performed. For the first time, a metabolic pathway synthesizing serine from a glycolytic precursor, 3-phosphoglyceric acid (3-PG), was demonstrated in human hair follicles and epidermis.

Serine biosynthesis from [^{14}C]-3-PG was demonstrated by incubating a human hair follicle homogenate in 0.1 M phosphate buffer, pH 7.5, in the presence of the cofactors glutamate, pyruvate, NAD, and dithiothreitol. The products of the reaction were [^{14}C]phosphoserine and [^{14}C]serine as demonstrated by paper and ion exchange chromatography. A boiled homogenate did not yield these products.

3-Phosphoglycerate dehydrogenase (3-PGDH), the rate-limiting enzyme in this pathway of serine synthesis, was assayed fluorometrically. Enzyme activity was linear with time, increased with the amount of follicular homogenate, was dependent on monovalent cations, and was inhibited by *p*-chloromercuribenzoate. K_m for PHPA (32.8 μM) and K_m for NADH (4.0 μM] were similar to other mammalian 3-PGDHs. 3-PGDH was in human and rat hair sheaths and bulbs, and in human and avian hair follicle-free epidermis. Activity decreased 4-fold from day 1 to day 12 of life in newborn rats; activity was unchanged by parenteral corticosteroids in adult mice.

The presence of this pathway of serine synthesis in keratinizing tissues means these tissues are not completely dependent on dietary or blood-borne serine for protein and nucleic acid synthesis.

Non-Plasminogen Activator-Mediated Cell Surface Protease Activity. PETER G. BURK, MICHAEL S. WERTHEIM, AND VICTOR B. HATCHER, Division of Dermatology, Department of Medicine and Biochemistry, Albert Einstein College of Medicine, Montefiore Hospital and Medical Center, New York, New York.

Plasminogen activator, an enzyme which converts serum plasminogen to plasmin, has been demonstrated to be on the cell surface and secreted into the media by many transformed and tumor cells in culture. There is evidence that plasminogen activator causes altered morphology and altered cellular mobility in cultured transformed cells. The present investigation was undertaken to learn whether cell surface protease activity other than plasminogen activator is present in normal and transformed cell lines and to compare this protease activity in normal and transformed cells. Cultured normal human fibroblasts, human melanoma cells, normal mouse epidermal cells, and transformed mouse epidermal cells were assayed for cell surface protease activity by washing 6 times with serum-free culture media and incubating with [^{3}H]acetyl casein in serum-free media at 37°C in room air under 7% CO_2. Aliquots were taken at 0, 60, 120, and 180 min to determine the percent of TCA-soluble [^{3}H]acetyl casein substrate. Appropriate control experiments failed to demonstrate secretion of protease or endocytosis of the casein substrate.

Our results indicate surface protease activity in all cell lines tested. The surface protease activity

was proportional to the cell number in each experiment. Approximately 1 to 6% of the total casein was degraded in a 3-hr incubation. High concentrations of ϵ-amino caproic acid, Trasylol and soybean trypsin inhibitor, plasminogen activator and plasmin inhibitors, did not reduce the cell surface protease activity. Transformed mouse epidermal cells had 3 to 4 times as much cell surface protease activity as did their normal counterpart.

SECOND WILLIAM MONTAGNA LECTURE

Ultrastructural and Lipid Biochemical Observations on the Genesis of Various Forms of Xanthomas. Frank Parker, Seattle, Washington.

AFTERNOON SESSION
Friday, April 30, 1976, 2:00 P.M.
POSTER SESSION

Role of Cyclic Nucleotides in Differentiation of Guinea-Pig Ear Epidermal Cells In Vitro. K. ASO, E. K. ORENBERG, D. I. WILKINSON, AND E. M. FARBER, Department of Dermatology, Stanford University, Stanford, California.

Guinea pig epidermal cells in culture were used to study the role of cyclic nucleotides in epidermal proliferation and differentiation. Cells were harvested from ear slices with trypsin (0.3%; 35 min; 37°C) and cultured in 60-cm plastic dishes (7–8 × 10^6 cells/dish) using MEM (fetal serum 9%) in 5% CO_2 for 14 days. Uptake of [^{3}H]thymidine (TdR), [^{3}H]leucine, and [^{3}H]histidine were observed daily by addition of 5, 1, and 1 μCi of each to different dishes for 1 hr followed by measurement of radioactivity incorporated into DNA or protein. DNA was assayed by the diphenylamine method. Daily, cGMP and cAMP were quantitated using 5 and 1 dishes, respectively, by radioimmunoassay. We have observed two distinct variations in cAMP levels. On day 4 of the first (growth) stage, there was a 40% decrease of cAMP concomitant with a peak of [^{3}H]TdR and amino acid incorporation. cGMP levels remained 0.064 ± 0.002 pmole/mg DNA over the first 6 days. After 6 days, differentiation became apparent as evidence by keratinization observed with acid fuchsin/aniline blue-orange G staining. During this second stage, [^{3}H]TdR uptake ceased, but amino acid incorporation increased and cAMP levels rose from 7.6 ± 3 pmol/mg DNA (day 5) to 35–40 pmol/mg DNA by day 12. Results suggest that higher levels of cAMP are associated with induction of differentiation and, conversely, low levels with proliferation.

Leukocyte Function in Cutaneous and Systemic Candidiasis. C. L. BERGER, A. N. DOMONKOS, AND M. SILVA-HUTNER, Department of Dermatology, Columbia University, New York, New York.

Susceptibility to Candida infection has been associated with immune dysfunction. Differences in immune function were assessed in 15 cutaneous candidiasis patients (CCP), 15 systemic candidiasis patients (SCP), and 15 controls by measurement of their polymorphonuclear (PMN) chemotaxis and phagocytosis, lymphocyte adherence, and serum antibody levels. Chemotaxis of PMN was measured in a Boyden chamber using *Candida albicans* blastospores as a chemotactic stimulant. Average chemotactic migration of PMN isolated from SCP was significantly greater (20.82 PMN migrated/HPF) than the migration of PMN isolated from CCP (5.98 PMN migrated/HPF) or controls (6.45 PMN migrated/HPF). Average phagocytosis of Candida cells was markedly decreased in PMN from SCP (39.43% phagocytosis) as compared to the CCP (71.40% phagocytosis) and controls (75.11% phagocytosis). Phagocytic killing of ingested yeasts was lower in both the SCP (2.45% of ingested yeast killed) and CCP (4.09%) than the controls (7.79%). Lymphocytes from SCP showed more adherent yeast cells (1.21 × 10^5 lymphocytes with one or more yeasts adherent/ml) than did lymphocytes from CCP (0.80 × 10^5/ml) or controls (0.49 × 10^5/ml). Positive precipitins and high agglutination titers were found in all groups. The increase in chemotaxis in SCP is apparently due to a serum factor that did not enhance phagocytosis. Increased chemotaxis and decreased PMN phagocytosis served to differentiate SCP from CCP and normals more accurately than antibody levels. Decreased phagocytosis in SCP may increase their susceptibility to Candida infection.

A Non-Nuclear Histone-Like Lysine-Rich Protein of Newborn Rat Epidermis. GOPAL M. BHATNAGAR AND IRWIN M. FREEDBERG, Department of Dermatology, Harvard Medical School and Beth Israel Hospital, Boston, Massachusetts.

We have previously described the isolation of a group of low-molecular-weight proteins from the keratohyaline granules of newborn rat epidermis. Among these the major protein (fraction 4) of 12,800 molecular weight has been further investigated. The protein has been isolated following DEAE-cellulose column chromatography, and antibodies to it have been produced.

Fraction 4 contains 18% lysine residues, migrates on polyacrylamide electrophoresis between calf thymus histone F1 and F3, and complexes with calf thymus DNA. Although the protein has characteristics of a histone, the histones of newborn rat epidermal nuclei do not contain the protein nor do ribosomes of epidermal or hair root cells.

Antibody raised to fraction 4 does not react with any other tissue of newborn or adult rat except for epidermis. A radioimmunoassay has been developed and has been used to quantitate the protein in animals of various ages. The protein is not present in fetuses 19 days or younger. It appears in 21-day fetal epidermis, increases in concentration at birth (22-day fetus), reaches maximum concentration 7 days later, and then declines rapidly. The adult rat epidermal concentration is minimal. Immunofluorescent studies have localized the pro-

tein to the superficial epidermal layers and have indicated that it is not a nuclear component. Our data indicate that this cytoplasmic protein has a role in the control of epidermal macromolecular metabolism.

T-Cell Inhibition by Immunoabsorbent Column-Purified IgG from Lupus Erythematosus Plasma. R. EDELSON, A. AHMED, F. FINKELMAN, A. STEINBERG, AND I. GREEN, Columbia University, New York, New York, and Naval Medical Research Institute, NIAID and NIAMD, National Institutes of Health, Bethesda, Maryland.

Plasmas from patients with systemic lupus erythematosus (SLE) contain antibodies reactive with thymus-derived (T) lymphocytes. To characterize the specificity and lymphocyte inhibitory properties of these antibodies, purified immunoglobulin fractions from 2 SLE patients were tested in several in vitro systems. These 2 patients were selected because their unfractionated plasmas diminished normal T-cell reactivity in mixed leukocyte cultures (MLC) by > 95%. Plasma was applied to Sephadex G-100 columns to which membrane fragments from hypotonically lysed leukemic T-cells or B (bone marrow-derived)-cell lymphoblasts had been previously attached after CNBr activation. Adherent antibody was eluted from the immunoabsorbent column with 0.1 M glycine HCl (pH 2.5) and then assayed for inhibitory activity in MLC. In addition, normal blood lymphocytes were stimulated with mitogen (phytohemagglutinin, concanavalin A, or pokeweed) or antigen (Candida, SK-SD or PPD). SLE antibody eluted from the T-cell immunoabsorbent column inhibited MLC (>99%) and antigen (>70% to all 3 antigens) responses. Control plasmas had no activity. Inhibition of blastogenic response to antigens was >50% in antibody dilutions as low as 1:25. No inhibition of the response to mitogens could be identified. The inhibitory antibodies did not bind to the B-cell column.

These results indicate that plasmas from the SLE patients contain specific antibodies which inhibit T-cell responses to soluble and membrane-associated antigens. Immunoabsorbent columns are valuable in the isolation and characterization of the anti-T-cell immunoglobulins.

Cell Proliferation Kinetics of Human Cutaneous Squamous Cell Carcinoma. A. FRIEDLAND AND G. WEINSTEIN, Department of Dermatology, University of Miami, Miami, Florida.

Cell proliferation kinetics of human epidermal squamous cell carcinoma (SCC) were studied to investigate basic tumor pathophysiology and to facilitate optimal therapy of widespread disease. Tumor cell proliferation kinetics were studied in vivo in 12 patients with cutaneous SCC. Standard autoradiographic techniques were used with intralesional [^{3}H]thymidine employed as a DNA marker. Serial biopsies from large or multiple SCCs were obtained 1 to 30 hr after [^{3}H]thymidine injections. A composite percent labeled mitosis (PLM) curve was constructed which was highly reproducible from patient to patient and from which S and G_2 phases of 12.5 hr and 6.9 hr, respectively, were determined. The labeling index of the proliferative cell population was found to be 35% ± 5%. From these data, the total germinative cycle of SCC was calculated to 35.8 hr.

Preliminary studies of SCC of the lung, head and neck, and cervix reveal remarkably similar labeling indices and cell cycles to the cutaneous SCC. This kinetic similarity may point to a common underlying pathophysiologic derangement controlling cellular proliferation in all SCCs. These cell proliferation kinetics may be applied to programming chemotherapy and/or radiation therapy for widespread cutaneous SCC as they have already been used for treatment schedules of SCC of the cervix and the head and neck.

Immune Complex Deposition in Leukocytoclastic Vasculitis. RICHARD G. GOWER, W. MITCHELL SAMS, JR, E. GEORGE THORNE, AND HENRY N. CLAMAN. Divisions of Clinical Immunology and Dermatology, University of Colorado, Denver, Colorado.

The pathogenesis of leukocytoclastic vasculitis is presumed to be secondary to the deposition of circulating antigen–antibody complexes in inflamed vessel walls. The present study was undertaken to determine the sequential development of immunologic and cellular events in induced vascular lesions. In 4 patients with acute vasculitis, lesions were induced by increasing vascular permeability by injection of 0.02 ml of histamine (.0275 mg/ml) into 4 sites of the skin of the lower legs. Biopsies were obtained for light and electron microscopy (EM) and immunofluorescence at 1, 4, 8, and 24 hr after injection. Zero time represents normal skin without injection. Results from 1 of the 4 patients were as follows, on a scale of 1 to 4+ (PP = palpable purpura, Mono = monocytes, Ig = immunoglobulins):

Time (hr)	PP	Ig	C3	Mono	PMNL	Rbc	Fibrin	EM deposits
0	0	1+	1+	1+	0	0	0	0
1	1+	4+	4+	1+	0	1+	0	2+
4	3+	2+	2+	3+	1+	2+	1+	2+
8	3+	1+	1+	2+	2+	3+	2+	1+
24	3+	1+	1+	1+	3+	4+	2+	0

The demonstration of early deposition of Ig, C3, and electron-dense deposits followed by cellular infiltration of vessel walls of induced lesions implies that Ig and probably circulating immune complexes are present prior to development of a lesion and supports the contention that deposition of

immune complexes within vessel walls is responsible for leukocytoclastic vasculitis.

RNA/DNA Ratio in Migrating Epithelium During Wound Healing. MICHAEL J. C. IM AND JOHN E. HOOPES, Division of Plastic Surgery, John Hopkins University School of Medicine, Baltimore, Maryland.

Previous studies in our laboratory revealed increased glycolytic and pentose phosphate pathway activity in migrating epithelium to provide certain basic substances necessary for increased biosyntheses. The present study of RNA/DNA and protein/DNA ratios was performed to identify any consequent increase in the synthetic products in the regenerating epithelium.

Measurement of RNA, DNA, and protein content in small segments of migrating epithelium was undertaken by application of microanalytical methods. Linear incisions (1 cm) were made on the backs of young guinea pigs. Skin biopsies were obtained from the wound sites and from contralateral control sites at intervals of 2, 3, 4, and 7 days following injury. Microdissection isolated relatively pure migrating or regenerated epithelium. RNA and DNA contents were measured by fluorometric methods utilizing 5–10 μg of freeze-dried epidermal tissue for RNA and 2–3 μg for DNA. Protein content was determined microspectrophotometrically utilizing 30 μg of freeze-dried tissue.

Regenerating epithelium exhibited increased RNA/DNA and protein/DNA ratios during the first 4 days of wound healing. Normal epidermis contains 2.27 mg total RNA/mg DNA. RNA/DNA ratios in the regenerating epidermis were 3.7, 4.1, 5.1, and 3.6 on days 2, 3, 4, and 7 after wounding, respectively. These findings coincide with previously measured biochemical parameters, i.e., enzyme activities and substrate levels during epidermal wound healing, and suggest an increased protein synthesis, including enzyme induction, secondary to trauma.

Isolation and Partial Characterization of Rat SRF (Skin Reactive Factor). BRIAN V. JEGASOTHY AND BYRON H. WAKSMAN, Yale University School of Medicine, New Haven, Connecticut.

Concentrated supernatants from normal or sensitized rat LNC (lymph node cells), cultured in serum-free medium with concanavalin A or antigen, respectively, contain a permeability factor (SRF) appearing by 12 hr and maximal from 24 hr on. SRF, when injected locally into rat flank skin, produces a permeability change in cutaneous vessels (blueing), which begins at 20 min, is maximal at 30 to 40 min, and fades by 6 hr. Blueing is not abolished in animals treated with inhibitors of histamine, serotonin, bradykinin, or SRS-A, or depleted of complement with cobra venom factor. Areas injected with SRF show perivascular accumulation of neutrophils in the lower dermis beginning about 1 hr after injection and peaking at 2½ to 3 hr. Partially purified SRF, tested on living rat cremaster muscle preparations observed microscopically (G Majno et al, Circ Res 21:823, 1967), causes a leakage of dye limited to small veins and venules, showing the same time course as skin blueing.

SRF appears to be a T-cell product. Its activity is destroyed by heating at 56°C for 30 min and by trypsin or periodate treatment, suggesting that it may be glycoprotein. Its molecular size (Sephadex G-100) is about 35,000 to 39,000. It does not react with rabbit antirat serum antibodies and is, therefore, probably not an immunoglobulin or other serum protein. The effect of SRF on venular permeability and migration of neutrophils suggests that it may have a biologic role in immune responses such as the Arthus phenomenon or cutaneous vasculitis.

Identification of a Human Melanoma Cell Line Containing Only a High K_m Cyclic Nucleotide Phosphodiesterase. L. E. KING, JR, S. S. SOLOMON, AND K. HASHIMOTO, Departments of Medicine, Dermatology and Research Service, Veterans Administration Hospital and University of Tennessee, Memphis, Tennessee.

Cyclic nucleotides play an important role in the metabolism of normal and neoplastic tissues. Cyclic nucleotide phosphodiesterase (PDE) is a key regulatory enzyme that controls levels of cyclic nucleotides by hydrolysis. The biochemical properties of PDE were examined in a cultured human melanoma cell line (KHm-1, wild type) we established 1½ years ago. PDE activity was found primarily (70–90%) in the 30,000 × g supernatant. The activity of the 30,000 × g extract was time, protein, pH, and Mg^{++} dependent. The PDE was sensitive to methylxanthines and various hormones and was inhibited by heavy metals and other noncyclic nucleotides. By kinetic analysis, only a single "high K_m" (1.5 mM) cyclic AMP (cAMP) PDE was detected. No PDE activity at high or low cyclic GMP (cGMP) concentration was detectable in any fraction tested. Isoelectric focusing of the 30,000 × g extract in polyacrylamide gels produced multiple bands that had cAMP PDE activity. Preliminary purification of the PDE by column chromatography indicated that the PDE has a molecular weight between 50,000 and 200,000. Also, a fraction that had PDE protein activator-like activity was present. The low total basal levels of cAMP PDE levels correlated well with the low basal levels of intracellular cAMP in this cell line.

Conclusions: In contrast to most normal tissues that contain multiple high and low K_m cAMP and cGMP PDEs, only a single high K_m cAMP PDE was identified and characterized in a human melanoma cell line. The homogenity of this cell line facilitates the purification of PDE and identification of genetic mechanisms controlling the production of cAMP and/or GMP PDE.

Effects of Colcemid on Epidermal Cell Migration. MORDECHAI KIRSH, K. FUKUYAMA, AND WILLIAM L. EPSTEIN, Department of Dermatology, University of California, San Francisco, California.

Previous studies indicate that microtubules may contribute to movement of cells in vitro. This study examines the effects of Colcemid, which dissolves microtubules, on epidermal cell migration in hairless mice. As a preliminary study, using 20 mice (25 gm), the optimal dose of Colcemid for arresting mitosis was found to be 0.1 mg/0.1 ml saline, with a peak metaphase index of 4.2% at 6 hr after intraperitoneal (ip) injection. [^{3}H]TdR (30 μCi) was injected ip in 6 mice. Colcemid (0.1 mg) was injected 16 hr later into 4 mice and 0.1 ml of saline into 2 mice. Ear skin specimens taken at various times were prepared for light microscopic autoradiography. By 21 hr after [^{3}H]TdR, labeled cells were found only in the basal layer (BL) of both treated and control mice. With time after injection, labeled cells appeared in the spinous layer (SL), attaining 2.9% of total epidermal cells in controls by 120 hr. In treated mice, however, only 1.8% of the cells moved into SL, and a higher percent of cells remained in BL, mostly not in arrested mitosis. The percent of labeled cells is summarized:

Hr after [^{3}H]TdR	Controls		Colcemid-treated	
	BL	SL	BL	SL
21	4.6	0	5.9	0
40	4.6	0.4	7.1	0
64	3.4	1.4	4.3	0.14
120	4.3	2.9	6.9	1.8

The results seem to indicate that Colcemid suppresses in vivo movement of epidermal cells as well as arresting mitosis, and suggests that microtubules may be involved in movement of epidermal cells.

Hapten Photochemistry: Characterization of 3,3′,4′,5-Tetrachlorosalicylanilide Photoproduct with Protein. IRENE E. KOCHEVAR (Introduction by L. C. HARBER), Department of Dermatology, Columbia University, New York, New York.

There has been considerable interest concerning complete antigen formation in photoallergy to 3,3′,4′,5-tetrachlorosalicylanilide (TSCA). Covalent bonding between hapten (TCSA) and protein has been demonstrated in vitro to result from light absorption by TCSA. To help characterize this photoadduct, a spectroscopic study of TCSA and two related molecules—one lacking halogens, salicylanilide (SA); one lacking an aniline ring, *N*-ethyl-3,5-dichlorosalicylamide (NEDS)—was performed. Fluorescence spectra were determined in the absence and presence of human serum albumin (HSA) in 0.01 M phosphate buffer (pH 7.4 containing 0.15 M NaCl and 5% ethanol). Fluorescence maxima of unirradiated 10^{-5} M solutions of TCSA, SA, and NEDS were observed at 440, 420, and 415 nm, respectively. Similar solutions kept in the dark but also containing 10^{-5} M HSA had fluorescence maxima at 432 nm (TCSA), 412 nm (SA), and 408 nm (NEDS). After irradiation of these solutions with 340–360 nm light, the photoadducts were separated from low-molecular-weight compounds by gel filtration chromatography. The emission maxima of the photoadducts were 455 nm (TCSA) and 414 nm (NEDS). No photoadduct was formed from SA, protein, and light. Energy transfer experiments demonstrated that TCSA, SA, and NEDS complex in the dark with HSA. However, formation of a covalent linkage between protein and either TCSA or NEDS requires light. These results indicate that chlorine substituents but not an aniline ring are essential for covalent photoadduct formation. Clinical observations parallel these findings; contact photodermatitis in man has required that salicylanilides are halogenated but that an aniline ring was not essential.

Photoconjugation of 3,3′,4′,5-Tetrachlorosalicylanilide to Proteins of Guinea-Pig Skin. Y. NAKAYAMA AND M. A. PATHAK, Department of Dermatology, Harvard Medical School, Boston, Massachusetts, and Shiseido Research Laboratory, Yokohama, Japan.

In photoallergic reactions of 3,3′,4′,5-tetrachlorosalicylanilide (T_4CSA), a light-induced covalent conjugation of T_4CSA (or its photoproducts) with proteins to form a complete allergen has been repeatedly hypothesized and even indirectly demonstrated. However, direct biochemical evidence of in vivo covalent linking of T_4CSA with epidermal proteins has been lacking. To demonstrate the in vivo photoconjugation of T_4CSA with proteins of epidermal-cell fractions and especially with microsomes, [^{14}C]T_4CSA (4,000 μg; 20 μCi) was applied to a 50-cm^2 area of guinea-pig skin and 90 min later the animals were irradiated (320–400 nm, 16–20 J/cm^2). The distribution of unbound and covalently conjugated moieties of T_4CSA in irradiated and nonirradiated skin specimens was examined after the isolation of the nuclear, mitochondrial, microsomal, and nonsedimentable soluble protein fractions of the epidermis. The protein-conjugated and nonconjugated T_4CSA moieties were detected by techniques such as counting of radioactivity, dialysis, precipitation and extraction, separation by column chromatography and gel electrophoresis, absorption and fluorescence spectroscopy, and enzymic digestion with DNase, RNase, and trypsin. The results indicate that: (1) T_4CSA covalently conjugates with epidermal proteins without irradiation; (2) conjugation of T_4CSA with proteins is enhanced (2 to 3 times) by UV irradiation; (3) the microsomal fraction showed the highest photoconjugation, followed in decreasing order by mitochondria, soluble proteins, and nuclear fractions; (4) the membranous components of microsomes and mitochondria also showed photo-

conjugation; and (5) the photoconjugated moities with epidermal proteins were 3,3',4',5-T_4CSA and 3,4',5-T_3CSA.

Schistosome Infection as a Model of Epithelioid Cell Granuloma (ECG). JAVIER NOGUERA, WILLIAM L. EPSTEIN, AND KIMIE FUKUYAMA, Department of Dermatology, University of California, San Francisco, California.

The granulomas produced by infection of hamsters with *Spirometra mansoni* were evaluated as a model for sarcoidosis. Development of ECG was studied by light microscopy. Two distinct patterns of cellular infiltrate were seen: (1) early (20–30 days) with an excess of eosinophils (eos) among lymphocytes (lymphs) and monocytes (monos); and (2) late (65–84 days) with emergence of epithelioid cells (EC) and plasmocytoid cells (Pl).

Percent cells						
eos	lymphs	monos	Pl	EC	Giant cell (GC)	
25.5	45.0	26.1	0.8	0.9	0.2	Early granuloma
4.6	15.8	45.6	11.0	22.2	0.6	Late granuloma

Electron microscopy revealed EC with vesicular and secretory endoplasmic reticulum which resembled sarcoidosis in organization. [^{3}H]TdR labeling at different stages in evolution of hepatic ECG were studied by autoradiography at 1 hr, 3 days, and 7 days post isotope injection.

[^{3}H]TdR	Percent labeling											
	Early granuloma						Late granuloma					
	eos	lymphs	monos	Pl	EC	GC	eos	lymphs	monos	Pl	EC	GC
1 hr	1.9	17.0	81.3	0.9	0	0.9	3.6	24.3	65.3	2.6	2.0	2.0
3 days	0.6	21.3	76.6	0	0.6	0.6	1.3	19.3	74.0	0	4.0	1.3
7 days	0	25.3	72.0	0	1.3	1.3	0	11.3	78.0	3.3	4.6	2.6

Uniquely, eos and Pl labeled, which suggests that they can divide in tissue and may modulate the formation of ECG. Large numbers of monos labeled early, with a tendency for EC to label later. These findings suggest that with some modification this model can be used to study granulomatous hypersensitivity.

Biphasic Effects of Tumor-Associated Antigens (TAA) on Host Immune Response (IMR). J. NORDLUND, R. CONE, R. K. GERSHON, AND A. ACKLES, Department of Dermatology and Pathology, West Haven Veterans Administration Hospital and Yale University, New Haven, Connecticut.

We reported that the IMR of DBA/2 mice against the syngeneic Cloudman melanoma is suppressed by excessively large or small amounts of TAA extracted from tumors. Intermediate doses of TAA are immunizing. To study further the ability of TAA to stimulate or suppress the IMR, we compared growth rates of tumors produced by inoculating into mice melanoma cells grown in tissue culture (TC-cells), 99% cell viability, to growth of tumors produced by injecting an equal number of live cells obtained from a tumor grown in a DBA/2 donor (An-cells) with 60% cell viability. Threshold dose for An-cells is 1×10^4 but for TC-cells is 5×10^5. The lag phase for the appearance of tumors from An-cells is 10 days shorter than the lag phase for TC-cells. Depriving the animal of its IMR (thymectomy and ALS at 6 weeks of age) markedly shortens the lag phase for tumors produced by TC-cells and slightly for An-cells, indicating that IMR, not metabolic factors, retards the appearance of tumors from TC-cells. In contrast, large doses of An-cells injected into mice (1×10^6) do not grow into tumors, but TC-cells grow readily. We suggest small amounts of TAA contaminating low doses of An-cells (1×10^5 or less) are immunosuppressive but larger doses of TAA (1×10^6 An-cells) are immunostimulating. Preliminary analysis of proteins from cell membranes on polyacrylamide gels indicates An-cells possess many more surface proteins with more rapid turnover than TC-cells. We hypothesize that labile TAA from membranes of Cloudman cells may enter the circulation of the host and, depending on concentration, suppress or stimulate the IMR against the incipient melanoma. This hypothesis could explain why some tumors escape immune surveillance.

Rates of Plasma Porphyrin Disappearance in Fluorescent vs Red Incandescent Light Exposure. M. B. POH-FITZPATRICK AND V. A. DELEO, Columbia University College of Physicians and Surgeons, New York, New York.

Although newer concepts of pathogenesis of phototoxicity in erythropoietic protoporphyria (EPP) depend on excess plasma protoporphyrin (PP), many symptomatic EPP patients have been reported with normal or only slightly elevated (0–5 μg/100 ml) plasma PP. Photolability of plasma PP has been observed (H van Gog and AA Schothorst, J Invest Dermatol 61:42, 1973). Rapid quantitative microfluorometric assay of porphyrins in minute quantities of plasma allowed more precise comparison of rates of porphyrin disappearance from

porphyric plasma, not only with variable light exposure, but also with variable storage temperatures. The $T_{\frac{1}{2}}$ of PP in EPP plasma was ≤30 min, and for uroporphyrin and coprophyrin in porphyria cutanea tarda (PCT) plasma was near 420 nm, when exposed to fluorescent room lighting (intensity at 405 nm ~ 7.2 $\mu w/cm^2$). The simple procedure of performing all blood drawing, processing, and assays in red incandescent lighting (intensity at 405 nm ~ 4.5×10^{-3} $\mu w/cm^2$) stabilized plasma porphyrin near 100% of original values for 5 days despite varying storage temperatures (4°, 28°, 37°C). Although no PP could be measured in EPP plasma exposed to fluorescent lighting after 24 to 48 hr, uroporphyrins and coproporphyrins remained measurable in fluorescent-exposed PCT plasma for several days. Plasma PP was then assessed in 20 EPP patients under red incandescent lighting. No negative values and only one value <15 μg PP/100 ml plasma was obtained (mean: 42; range: 11–100 μg PP/100 ml plasma).

These data suggest that many normal or trace values for plasma PP reported in symptomatic EPP patients may reflect effects of even brief exposure of plasma specimens to ambient lighting.

Lucio's Phenomenon: An Immune Complex Complication of Lepromatous Leprosy. F. P. Quismorio, T. H. Rea, N. E. Levan, and G. J. Friou, Department of Medicine, University of Southern California, Los Angeles, California.

Lucio's phenomenon is an acute reaction in diffuse lepromatous leprosy characterized by cutaneous infarctions secondary to necrotizing vasculitis. Four cases of Lucio's phenomenon were studied to elucidate the pathogenesis of this complication of leprosy.

Circulating immune complexes were found in 4 patients by Raji cell test. Cryoglobulins ranging from 1.2 to 34 mg% were also detected. These were "mixed" type consisting of IgG, IgM, IgA, C1q, C3, and C4. In 2 patients, IgM rheumatoid factor activity was present in the cryoglobulins. Hypocomplementemia was noted in 1 case. By direct immunofluorescence, deposits of IgM, IgG, and C3 were found in blood vessel walls and interstitium of the dermis. Cryostat sections of skin were incubated with sheep red blood cells (SRBC) sensitized with IgG anti-SRBC and IgM anti-SRBC coated with mouse complement to characterize infiltrating lymphoid cells. Cells with complement receptors were found but most of the cells did not react with either reagent, suggesting that these may be T-lymphocytes. Our data suggest that Lucio's phenomenon, like erythema nodosum leprosum, may be an immune complex disorder.

Immunopathology of Oral Inflammatory Diseases. Roy S. Rogers III and Robert E. Jordon, Departments of Dermatology and Immunology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

Direct immunofluorescent (DIF) testing has provided a valuable diagnostic and investigative tool for the study of cutaneous diseases. During the past 2½ years we have applied DIF to confirm clinical diagnoses and to provide insight into the pathogenesis of bullous, erosive, and ulcerative diseases affecting the oral mucosa.

Patients with oral mucosal diseases for which a diagnostic biopsy specimen was indicated were studied. Perilesional tissue was selected for biopsy as this specimen was more likely to provide diagnostic material than lesional tissue. Specimens were obtained for both histopathologic and immunopathologic study, the latter were frozen immediately and were studied for or stored at −70°C until study. DIF investigations were conducted using antisera to IgG, IgA, IgM, C1q, C4, C3, properdin, Factor B, and fibrin.

The study involved 124 oral mucosal biopsy specimens. There were no false positive results. Diagnostic results were noted in 15/15 pemphigus vulgaris, 3/3 bullous pemphigoid, and 19/22 cicatricial pemphigoid biopsy specimens. Support for the concept that some patients with desquamative gingivitis fall into the pemphigoid spectrum of diseases was noted. DIF results were suggestive of lichen planus in 24/33 biopsy specimens, but similar results were noted in 6/42 biopsy specimens of miscellaneous oral inflammatory diseases.

DIF testing is an excellent diagnostic tool and provides insight into the pathogenesis of oral bullous, erosive, and ulcerative diseases.

Depressed Leukocyte Chemotaxis and Lymphocyte Transformation in Severe Atopic Dermatitis. J. L. Rogge and J. M. Hanifin, Department of Dermatology, University of Oregon Health Science Center, Portland, Oregon.

Altered immune function, including relative skin test anergy and unusual susceptibility to infectious agents, has been well documented in patients with atopic dermatitis. We assessed various in vitro parameters of cell-mediated immunity in atopic individuals to determine whether leukocyte defects might underlie these immune deficiencies.

Using a modification of Boyden's technique, we found polymorphonuclear leukocyte (PMN) chemotaxis to be markedly depressed in each of 7 erythrodermatous atopic dermatitis patients tested. Simultaneous evaluation of monocyte chemotaxis and lymphocyte response to phytohemagglutinin (PHA) in 4 of these individuals revealed additional defects:

	PMN chemotaxis	Monocyte chemotaxis	Lymphocyte PHA response
Average % of normal control values	11.3%	25.5%	18.6%
p value	<.001	<.001	<.005

PMN chemotaxis examined during clinical remission in 3 patients demonstrated significant improvement in cell migration. Normal PMNs incubated in plasma from 4 of these patients showed a mean 50% depression of chemotaxis. Serum IgE levels were consistently elevated in all atopic dermatitis patients, yet only the severe erythrodermatous ones had associated leukocyte defects. Less severe atopic patients and individuals with generalized erythroderma from other causes served as controls.

Atopic plasma inhibition of normal PMN chemotaxis suggests an inhibitory factor may be responsible for the simultaneous dysfunction of 3 major host defense cells during severe flare of atopic dermatitis.

Antigen-Bearing Langerhans Cells in Dermal Lymphatics and in Lymph Nodes. INGA SILBERBERG-SINAKIN, G. JEANETTE THORBECKE, RUDOLF L. BAER, STANLEY A. ROSENTHAL, AND VERA BEREZOWSKY, Departments of Dermatology and Pathology, New York University School of Medicine, New York, New York.

Studies were done with ferritin (Fe), an antigen visualizable by electron microscopy, to ascertain whether Langerhans cells (LC) can take up antigen in skin and carry it to lymph nodes (LN). We compared events which ensue in skin and LN after intracutaneous injection of 5 or 30 μg of Fe in 4 guinea pigs (GP) which had been passively sensitized to Fe by lymphoid cell transfer and in which we elicited delayed hypersensitivity reactions to Fe. Four GP passively sensitized to peroxidase by lymphoid cell transfer and 4 normal GP served as controls. After intradermal challenge with Fe, in control GP, and to an apparently greater degree in Fe-sensitive GP, Fe-containing LC were seen 4 hr after challenge in the marginal sinus and cortex of draining LN, as well as 3 hr after challenge in a dermal lymphatic. Fe was found in LC on the surface and in membrane-bound inclusions. LN from unchallenged normal GP contained rare LC, none of which had Fe. LC, similar to macrophages, were radioresistant to 800 rad in whole-body-irradiated GP. Thus, LC in normal and sensitized GP pick up Fe in skin and circulate to draining LN, carrying out a function analogous to macrophages. These and other previous observations (juxtaposition of LC and lymphocyte-like cells in skin and LN draining allergic contact reactions) strongly suggest that LC exhibit antigen to lymphocytes, both in skin and LN in cell-mediated cutaneous hypersensitivity reactions. The role of skin as an organ participating in immunologic reactions, especially in delayed hypersensitivity, perhaps can be explained by the relaying of information from skin to lymphoid tissues via LC.

Isolation of an Actin-Like Protein from Epidermal Tumors and Cultured Epidermal Cells. P. STEINERT, G. PECK, S. YUSPA, J. MCGUIRE, AND A. DITASQUALE, Dermatology Branch (PS, GP) and Experimental Pathology Branch (SY), National Cancer Institute, National Institutes of Health, Bethesda, Maryland, and Dermatology Department (JMcG, ADiT), Yale University School of Medicine, New Haven, Connecticut.

Microfilaments, cytoplasmic constituents of many motile cell types, are composed of an actin-like protein (ALP) that is necessary for the cellular movement. ALP has been found in a variety of invasive cancers and cells in vitro. Using immunofluorescent methods, McGuire, Lazarus, and DiTasquale (to be published) have found ALP in cultured bovine epidermal cells and isolated it from normal bovine epidermis. We have now used SDS gel electrophoresis to investigate the presence of ALP in 8 M urea extracts of epidermal tumors and several epidermal culture systems. Whereas ALP represents less than 1% of the total protein of normal human, bovine, and murine epidermis, it increases to about 10% in primary cultures derived from each of these tissues and 75% in subcultured bovine epidermal cells. In vitro, the normal complement of keratin bands on gels was present in bovine epidermis but not in the other cell types. ALP constitutes about 25 to 50% of the total protein extracted from human basal cell carcinomas and chemically induced mouse papillomas and carcinomas. These tissues also displayed altered keratin bands on gels. The abundance of ALP in mouse epidermal cells in vitro and chemically induced mouse epidermal tumors allowed its isolation using methods developed for muscle actin. The isolated ALP undergoes G- to F-actin transformations in vitro, can form actomyosin with mouse muscle myosin, and has physicochemical properties identical to those of mouse muscle actin. ALP may serve as a useful biochemical marker for studies of epidermal tumors and in vitro carcinogenesis.

Ultrastructural Studies of the Nuclei in Premitotic (S-Phase) and Repair DNA Synthesis Following UV-B Injury. H. SUZUKI, K. FUKUYAMA, J. H. EPSTEIN, AND W. L. EPSTEIN, Department of Dermatology, University of California, San Francisco, California.

This report concerns ultrastructural changes in nuclei synthesizing DNA as studied by cytochemical techniques. Guinea-pig ears were used, since groups of epidermal cells undergo DNA repair at 1 hr and S-phase at 24 hr after UV-B irradiation (4.48×10^7 ergs/cm^2). [^{3}H]TdR was injected intradermally into UV-B irradiated sites, and specimens obtained 1 hr later were fixed in 3% glutaraldehyde, embedded in glycolmethacrylate, and prepared for autoradiography. Areas of the epidermis containing more than 80% of cells in DNA synthesis were selected for each time period and cut at 600 Å. The sections were stained with uranyl acetate and lead citrate, and consecutive sections were incubated with 0.01% pronase and 0.5% RNase before staining in order to observe DNA.

In cells undergoing DNA repair, unlike the

normal accumulation along the nuclear membrane, the zone of DNA became discontinuous and DNA was scattered throughout the entire karyoplasm as small aggregates and fine filaments. Nuclei in S-phase showed essentially the same change, but quantitatively the disappearance of DNA from the nuclear membrane and distribution in the karyplasm became much greater. These changes were not seen in specimens not treated with enzymes.

The findings indicate that distinct ultrastructural changes occur in distribution of DNA in the nucleus during DNA synthesis, and they are qualitatively similar for both S-phase and repair synthesis.

Biologic and Biochemical Actions of Methotrexate in Psoriasis. G. WEINSTEIN, A. FRIEDLAND, AND J. MCCULLOUGH, Department of Dermatology, University of Miami, Miami, Florida.

As part of a program to understand why topical methotrexate (MTX) is ineffective in psoriasis, we examined whether the biologic effects of MTX in psoriasis occur by direct drug action on the psoriatic plaque or are mediated by systemic action at a site distant from skin. Ten patients were injected intralesionally (il) with varying quantities of MTX (0.0001–0.25 mg) and biopsied serially. The same biologic effects seen with systemically administered MTX were found: a comparable decrease in mitotic activity and production of "MTX-damaged cells." These effects appeared 2 hr post injection and were dose related. The data suggest that MTX acts directly on the psoriatic plaque rather than systemically at a distant site.

The biochemical actions responsible for the biologic effects of MTX in psoriasis were also studied. Four psoriatic patients were injected il with MTX simultaneously with either thymidine, leucovorin, uridine, or saline and biopsied serially. The thymidine (10^{-5} M) administration protected against the biologic effects of MTX, described above, in all patients. Leucovorin provided only partial protection, while uridine and saline had no influence on the MTX effects. The data suggest that these biologic effects of MTX in psoriasis are mediated by the depletion of the thymidine pool rather than by other biochemical actions.

Defective Monocyte Chemotaxis in Mycosis Fungoides. W. L. WESTON, D. L. NORRIS, L. E. SEITZ, L. E. GOLITZ, AND J. L. AELING, Division of Dermatology, University of Colorado Medical Center, Denver, Colorado.

Fifteen patients with histologic and clinical mycosis fungoides had depressed ($p < 0.01$) monocyte chemotaxis (mean ± SD = 4.3 ± 3.4) when compared to 35 healthy controls. Only 2 of 15 patients demonstrated chemotaxis values within 2 SD of normal controls.

Monocyte chemotaxis was performed on suspensions of monocytes obtained from peripheral blood by the Ficoll-Hypaque method in modified Boyden chambers assayed in triplicate. A single endotoxin-activated AB-negative serum was used as the standard attractant. Monocyte phagocytosis and killing of Staphylococcus 502A was normal in 11 patients, demonstrating a selective defect in monocyte chemotaxis. Nine patients were untreated at the time of study but did not differ in depressed chemotaxis from 4 treated with topical nitrogen mustard nor 2 patients post radiotherapy.

	Mean % cells responding	Patient	Mean % cells responding	Patient	Mean % cells responding	Patient	Mean % Cells responding
A	3.9	E	5.1	I	3.0	M	1.1
B	3.9	F	2.2	J	5.1	N	1.4
C	4.5	G	3.8	K	4.7	O	10.2
D	7.6	H	5.5	L	2.2	Controls	11.6 ± 6.0

Defective monocyte chemotaxis represents an unrecognized defect in patients with mycosis fungoides that may explain their susceptibility to frequent cutaneous infections and poor host response to invading microorganisms resulting in septicemias and other life-threatening infections.

Inhibition of Collagen Formation by Proline Analogues: Degree of Replacement and Specificity of Action. JOUNI UITTO AND DARWIN J. PROCKOP, Department of Dermatology (JU), Washington University School of Medicine, St. Louis, Missouri, and Department of Biochemistry (DJP), CMDNJ-Rutgers Medical School, Piscataway, New Jersey.

Several proline analogues, when incorporated into procollagen, prevent the molecules from folding into triple-helical conformation; the nonhelical protein-containing analogue is unable to form extracellular fibers. In this study, chick tendon fibroblasts synthesizing procollagen were incubated with 0.3 mM α,α'-dipyridyl and 1.53 mM *cis*-4-hydroxy-L-proline or 1.50 mM *cis*-4-fluoro-L-proline. The unhydroxylated procollagen polypeptides were isolated by digestion with highly purified bacterial collagenase, and the content of the analogues and proline in these peptides was assayed. The results demonstrated that substitution of about 1.5 of the imino acids in procollagen was sufficient to prevent the helix formation. In further experiments, chick aorta cells, which are known to synthesize and secrete both procollagen and elastin, were incubated with 1.53 mM *cis*-4-hydroxy-L-proline, 1.0 mM azetidine-2-carboxylic acid, or 0.22 mM 3,4-dehydro-DL-proline. No intact triple-helical procollagen was recovered in the medium. Elastin polypeptides, however, were synthesized and secreted at the normal rate. These observations support previous suggestions that proline analogues may function as specific inhibitors of procollagen synthesis and they may prove useful in preventing the excessive accumulation of collagen in tissues in clinical situations.

MORNING SESSION
Saturday, May 1, 1976, 9:00 A.M.

BUSINESS AND EXECUTIVE SESSION
John S. Strauss, Boston, Massachusetts, Presiding

SCIENTIFIC SESSION
Adolph Rostenberg, Jr., Chicago, Illinois, Presiding

Experimental Cutaneous Candidiasis: Role of the Stratum Corneum. THOMAS L. RAY, DIANE H. BAKER, AND KIRK D, WUEPPER, Department of Dermatology, University of Oregon Health Sciences Center, Portland, Oregon.

Candida species *albicans* and *stellatoidea* but not *tropicalis*, applied under occlusion, penetrate intact stratum corneum (SC) and induce epidermal microabscesses in rodents (Clin Res 23:230A, 1975). To evaluate the role of SC, 1- to 4-day-old white/Swiss mice were injected with 2 μg of the staphylococcal exotoxin, epidermolysin, to induce epidermal separation. Saline or Sabouraud broth suspensions of the above Candida species, containing 1×10^6 particles, were injected into this epidermal split below the SC. Leukocyte migration into the epidermis and cleft occurred within 24 hr when either viable or heat-killed Candida species were used. All viable species tested produced hyphal invasion limited to the epidermis. Injection of saline, Sabouraud broth, or epidermolysin alone, resulted in no leukocyte accumulation.

Since viable or heat-killed Candida activate complement and induce leukocyte migration, we studied leukocyte responses in B10/D2/0 mice deficient in the fifth component of complement, C5. Neither viable nor heat-killed *C. albicans* evoked leukocyte accumulation. However, viable *C. albicans* produced unrestricted hyphal proliferation throughout the cutis and subcutis. Similar results were obtained in mice treated with cobra factor (CoF). CoF did not inhibit growth of *C. albicans*.

Intact SC is an effective barrier to *C. tropicalis* but not *C. albicans* or *C. stellatoidea*. Pathogenesis is associated with penetration of SC, and contact of viable or heat-cilled Candida elements with viable epidermis. Confinement of hyphal projections by cellular inflammatory response requires C5 or later-acting complement components.

Trichophytin Reactions—Further Investigations on Cutaneous Basophilic Hypersensitivity. J. H. GREENBERG, S. KERBS, R. FIELDS, AND R. D. KING, Letterman Army Institute of Research, San Francisco, California.

Infections of inexperienced guinea pigs with *Trichophyton mentagrophytes* sensitizes these animals to isolated trichophytin antigen. Skin tests with this antigen on immune guinea pigs have the following characteristics: onset at 1 hr, maximum size and erythema at 24 hr (fading over the next 48 hr), and minimal induration. Histopathology shows a large number of basophils in the dermal infiltrate which makes this skin test reaction consistent with cutaneous basophilic hypersensitivity (CBH). Studies were performed to delineate the influence certain immunologic manipulations would have on this reaction. CBH could be passively transferred from experienced to inexperienced animals by passage of lymphocytes. Immunization with dermatophyte hyphae plus complete Freund's adjuvant gave classical delayed hypersensitivity. Cyclophosphamide was given to a group of animals prior to an infection and these were compared to infections in animals not receiving any chemotherapeutics. The cyclophosphamide animals developed classic delayed hypersensitivity to trichophytin whereas the control animals all had cutaneous basophilic hypersensitivity to this antigen. Both groups ran the same infection course and there was no difference in either group on reinfection. Animals reacting with classical delayed hypersensitivity as measured by trichophytin did not differ in their immunity to dermatophyte infections from animals reacting with cutaneous basophilic hypersensitivity.

SPECIAL LECTURE

On the Mechanism of Phagocytosis. THOMAS P. STOSSEL, Boston, Massachusetts.

Basement Membrane Zone (BMZ) Antibody Activity after Successful Treatment of Bullous Pemphigoid (BP) Patients. A. RAZZAQUE AHMED, JOHN C. MAIZE, AND THOMAS T. PROVOST, Department of Dermatology, State University of New York at Buffalo, Buffalo, New York.

This longitudinal study employs direct (DF) and indirect (IF) immunofluorescent techniques to determine skin and serum BMZ antibodies before, during, and after therapy of BP. Thirty-six patients were followed for a mean of 18 months (range 3–33) with DF and IF studies every 4 months. All patients initially had positive DF. Seven patients failed to demonstrate serum BMZ antibodies. Seven patients with localized disease spontaneously cleared. Fourteen BP patients were treated with corticosteroids and 15 with steroids plus azothioprine (Imuran). All patients demonstrated a fall of serum BMZ antibody titers while on therapy. Fourteen clinically well patients on no therapy for a mean time of 25 months have no serum or skin BMZ antibodies, but 10 relapses in 7 patients were associated with reappearance of skin and serum BMZ antibodies (50%). The data suggest that: (1) approximately 20% of BP patients have localized disease that clears spontaneously, (2) more than 50% of BP patients after initial successful therapy have a prolonged clinical remission in which no BMZ antibody can be detected, (3) clinical BP activity and its recurrence are associated with positive DF (in 9 instances purely C3), and (4) azothioprine plus steroids appear superior to steroids alone in the management of BP patients.

Herpes Gestationis: Immunoperoxidase Localization of C3 and Ultrastructural Observations of Normal and Lesional Skin. H. YAOITA, M. GULLINO, AND S. I. KATZ, Dermatology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

Ultrastructural localization of C3 deposition in the skin of 2 patients with herpes gestationis was determined by using a recently described peroxidase-antiperoxidase multistep technique (K Holubar et al, J Invest Dermatol 64:220, 1975). The reaction products were seen throughout the entire lamina lucida. This localization of C3 is similar, though not identical, to that of the immunoglobulin and C3 bound in skin of patients with bullous pemphigoid.

In addition, ultrastructural observations of the skin of the same patients showed the most remarkable changes in the normal-appearing skin to be the destruction of the basal cell membranes at their dermal side, localized cytoplasmic dissolution, and intracellular edema unaccompanied by inflammatory cells. Early, nonvesicular lesions showed basal cell degeneration and dermal inflammatory cells. Necrosis and loss of basal cells occurred in the next stage which resulted in microvesicles in which collagen or, in many instances, an intact basal lamina composed the vesicle base. In the later blister state, the basal lamina was usually lost but sometimes was found at the blister base with cell organelles and basal cell fragments attached to it. Occasionally fragments of all components of the basement membrane zone were seen in the blister.

It is suggested that damage of basal cell membranes at their dermal side leads to the destruction of basal cells with the subsequent protrusion of epidermal and junctional substances into the dermis. This may result in inflammatory cell infiltration and blister formation.

Effects of the Pemphigus Autoantibody on Human Epidermal Cells in Organ or Suspension Culture. JOHN R. SCHILTZ AND BENO MICHEL, Division of Dermatology, Case Western Reserve University, Cleveland, Ohio.

Normal human skin was maintained as organ cultures in Ham's F-10 medium or F-10 + normal human IgG (NIgG) for several days with good perservation of the epidermal cells. When purified IgG from pemphigus sera (PIgG) was added to cultures, however, progressive epidermal changes were seen. By 72 hr, extensive suprabasilar acantholysis occurred in which the acantholytic cells were indistinguishable from those seen in biopsies of pemphigus vulgaris. Direct immunofluorescent staining of these explants showed that maximal binding of the pemphigus IgG occurred in the intercellular cement substance (ICS) prior to the onset of acantholysis. When pemphigus serum was fractionated on a DEAE-cellulose column, three major IgG-containing peaks (IgG subclasses?) were eluted which bound to the epidermal ICS and caused acantholysis in culture. The complement system did not play a role in the antibody-induced acantholysis since heating the F-10 + PIgG medium for 1 hr at 58°C did not destroy the acantholytic activity. Autoradiographic experiments showed that after 2 days in culture with PIgG the rates of synthesis of RNA and protein in the suprabasilar cells were reduced to less than 10% of the NIgG or F-10 controls, whereas the basal cells were only slightly affected. Studies using epidermal cell suspensions showed that after an 18-hr incubation with a ^{3}H-amino acid mixture, protein synthesis was inhibited 65% by the PIgG fraction. This effect is both time and antibody-concentration dependent.

This work supports the proposal that the pemphigus autoantibody(s) interacts with the suprabasilar epidermal cell to initiate and possibly sustain the process(es) of acantholysis.

AFTERNOON SESSION

Saturday, May 1, 1976, 2:00 P.M.

John S. Strauss, Boston, Massachusetts, Presiding

SIXTEENTH ANNUAL HERMAN BEERMAN LECTURE

Mechanism of Steroid Hormone Regulation of Gene Expression. BERT W. O'MALLEY, Houston, Texas.

Tissue Profiles of Carcinogen Metabolism Following Skin Application of Chemical Inducers. D. R. BICKERS, Department of Dermatology, College of Physicians and Surgeons, Columbia University, New York, New York.

Drug-metabolizing enzymes are found primarily in the liver and to a lesser extent in extrahepatic tissues such as skin. These microsomal enzymes can metabolize polycyclic hydrocarbon carcinogens including benzo[a]pyrine (BP) into a variety of products including hydroxylated BP (OHBP). Some environmental pollutant chemicals are carcinogenic and may induce drug metabolism in the liver and other tissues. This study assessed total-body drug metabolism using the carcinogen-metabolizing enzyme, aryl hydrocarbon hydroxylase (AHH), as a prototype. Neonatal rats were treated with skin application of either the environmental pollutant, polychlorinated biphenyls (PCB) or the polycyclic hydrocarbon carcinogen BP (25 mg/kg/day × 6). Controls received vehicle alone. Using a fluorometric assay to quantitate OHBP, AHH activity was determined in epidermis, dermis, liver, lungs, kidney, and the remaining whole animal. In controls, 85% of the total-body AHH activity (1.39×10^{5} pmoles OHBP) was in liver, with lesser activity in skin (3.2%), lung (2.7%), and kidney (6.7%). After PCB treatment there was 5- to 10-fold induction of AHH activity in each tissue (total = 1.44×10^{6} pmols OHBP). The liver of PCB-treated rats had 58% of total-body AHH; skin activity increased to 35% with lesser amounts in lung (0.6%) and kidney (0.9%). BP-treated rats

also showed a 5- to 10-fold increase in total-body AHH (1.34×10^6 pmoles OHBP). Liver contained 60% of total-body AHH and skin 18%, with lesser amounts in lung (2.1%) and kidney (5.3%). These studies indicate that drug-metabolizing enzymes present in the skin can contribute significantly to the body's metabolic response to environmental chemicals.

Human Melanoma: Biologic and Ultrastructural Studies In Vitro. T. KANZAKI, K. HASHIMOTO, AND D. BATH, Department of Medicine, Division of Dermatology, Veterans Administration Hospital and University of Tennessee, Memphis, Tennessee.

Human melanoma has not been investigated extensively in vitro. We established and maintained in vitro a cell line of human melanoma, KHm-1, wild type, for 1½ years. This cell line fulfills the criteria for in vivo and in vitro malignancy: loss of contact inhibition, cell agglutination by concanavalin A, cell growth on fibroblast monolayers and in soft agar, and animal tumorigenicity in nude mice. Karyotypes showed two distinct marker chromosomes, one in A group and the other in B group. The population doubling time was 34 hr and plating efficiency was 45%. The cell shape varied from round, spindle, dendritic, to multinucleated giant (MNGC) cells. Time lapse photography revealed that MNGC were produced by nuclear replication without cell separation. Paramyxovirus-like inclusions were found in these MNGC as well as single nucleated cells. Spindle cells became dendritic in 24 hr by treatment with dibutyryl cAMP, theophylline, or serum-free medium. The dendritic cells rounded up 3 min after removing theophylline or adding serum and returned to their spindle shape in 24 hr. These three different-shaped cells differed primarily in the distribution and quantity of 60 Å submembranous microfilaments (SMMF). The presence of SMMF appeared to be inversely correlated with the morphologic differentiation in this cell line: SMMF were most prominently and evenly distributed in round cells and the spindle cells showed many fewer SMMF. SMMF were not observed in dendritic cells. The amount of SMMF is intimately related to the malignancy since round cells satisfied most indicators of cell malignancy such as low level of cAMP and weak adhesion to substratum.

A Potent Inhibitor of Normal and Transformed Cell Growth Derived from Contact-Inhibited Melanocytes. GEORGE LIPKIN AND MARGARETE E. KNECHT, Department of Dermatology, New York University Medical Center, New York, New York.

Melanocyte contact inhibitory factor (MCIF) restores contact inhibition of growth (C.I.) to malignant melanocytes of man, mouse, and hamster (Proc Natl Acad Sci USA 71:849, 1974; Schweiz Med Wochenschr 105:1360, 1975). Studies of tissue specificity showed that MCIF (25–100 μg/ml) effectively (20–96%) inhibits growth of benign and malignant cells of ectodermal, mesodermal, and endodermal derivation, including neuroblastoma, glioma, neurinoma, mammary carcinoma, rhabdomyosarcoma, colon carcinoma, fibroblasts, and epidermis in a dose-dependent, nontoxic, reversible manner. Neuroblastoma cells exhibited pronounced neurite differentiation. High concentrations (100–400 μg/ml) which merely inhibited growth of fibroblasts or epidermal cells, were lethal to several neoplasma. Pulse-labeling experiments showed MCIF-treated melanoma cells to be contact-inhibited in G_1 phase of the cell cycle, mimicking prototypical contact-inhibited diploid fibroblasts. MCIF-induced C.I. was not altered by epidermal growth factor (EGF), but was abolished by inhibitors of microtubules of microfilaments, and such loss was not prevented or reversed by dbcAMP (10^{-3} to 10^{-9} M). Neither dbcAMP nor 250K protein reproduced the morphologic effects of MCIF. Human and murine (3T3) fibroblasts, and human epidermal cells (BE line) produce a protein with mobility on SDS gels identical to that of MCIF; this protein is greatly reduced in cultures of viral-transformed fibroblasts (SV40 3T3). MCIF, a growth regulatory macromolecule associated with contact-inhibited cells, is a potent inhibitor of normal and neoplastic cell growth whose effects transcend tissue and species barriers.

Prostaglandin E Levels in Sunburned Mammalian Skin. DIANE SEKURA SNYDER, Department of Dermatology, University of Miami, Miami, Florida.

Exposure of mammalian skin to ultraviolet light from 290–320 nm (UV-B) produces a biphasic erythema response. In this study the level of prostaglandin E (PGE) in guinea-pig skin exposed to UV-B was measured as a function of time after irradiation and as influenced by indomethacin (IM). The PGE levels were related to the development of erythema.

Total lipids were extracted from skin biopsies with $CHCl_3$:MeOH (2:1), fractionated into classes by silicic acid column chromatography, and assayed for PGE using a radioimmunoassay procedure.

The level of PGE in unirradiated normal skin was determined to be 106.8 ± 15.0 ng/gm tissue or 690 ± 69 ng/mg protein (mean ± SEM, n = 14). PGE levels in UV-B-irradiated skin increased during the first 4 hr after UV-B in a manner which paralleled the development of the delayed phase of erythema. By 4 hr after UV-B the PGE levels had reached 3 times the value of control skin and had returned to preirradiation values by 48 hr. Erythema was still present at 48 hr.

A single topical application of 2.5% w/v IM decreased both erythema and PGE levels within 1 hr of treatment. PGE levels stayed depressed to near normal throughout the next 48-hr period.

Erythema which had been markedly reduced by IM began to increase at 24 hr and did not respond to additional applications of IM.

The labeling index in UV-B-exposed skin showed an inverse relationship to the PGE content. Although treatment of sunburned skin with IM decreased PGE levels to normal, it has no effect on the labeling index.

These results show that PGE plays a major role as mediator or potentiator of the delayed phase of UV-B-induced erythema. Involvement of PGE in UV-B-induced alterations in DNA synthesis was not obvious.

Cytologic and Metabolic Effects of Prostaglandins on Rat Skin. AUREL P. LUPULESCU, Department of Dermatology, Wayne State University, Detroit, Michigan.

Prostaglandins are cellular mediators which intervene in the evolutions of cutaneous inflammatory lesions and wound healing. However, their intimate mechanism at cellular level is still unknown. To clarify this mechanism, we investigated the effects of prostaglandins (PGE_1, PGE_2, and $PGF_{2\alpha}$) on DNA, RNA, protein, and collagen synthesis in rat skin using scintillation counting, autoradiography, electron microscopy, and scanning electron microscopy. Prostaglandins were injected intramuscularly at a dose of 150 μg every other day. Radioactive precursors were injected intraperitoneally at a dose of 1 μCi/gm body weight. Radioactivity measurements of skin specimens revealed that PGE_1 and PGE_2 increased the incorporation of [^{3}H]thymidine, and [^{3}H]uridine, and [^{3}H]leucine at 1 hr, which moderately declined by 5 to 24 hr. Proline incorporation was increased at 24 hr. $PGF_{2\alpha}$ decreased the incorporation of all radioactive precursors. Electron microscopy revealed that tested prostaglandins markedly affect the ultrastructural morphology of epidermal cells and fibroblasts. PGE_1 and PGE_2 increased polysome population, tonofibrils, size of nuclei and nucleoli, keratohyaline granules, and cell mobilization with enlarged intercellular spaces and cytoplasmic projections, whereas $PGF_{2\alpha}$ induced an advanced cytolysis, cell disintegration, and phagolysosomes. Fibroblasts are hypertrophied with collagen fibers. Scanning electron microscopy revealed intense collagen synthesis with more compact hypertrophied collagen fibers, whereas they appeared as a loose network of collagen fibers and fibrils in control rats. These findings demonstrate that prostaglandins E_1 and E_2 stimulate DNA, RNA, protein, and collagen synthesis in the rat skin, whereas $PGF_{2\alpha}$ inhibits it.

Inhibitor(s) of Prostaglandin Synthesis in Psoriatic Plaque. NEAL S. PENNEYS, VINCENT A. ZIBOH, JONATHAN T. LORD, AND PAUL E. SIMON, Department of Dermatology, University of Miami, Miami, Florida.

During coal-tar and ultraviolet-light therapy, a white ring (Woronoff) frequently develops in the normal skin adjacent to psoriatic plaques while uninvolved skin develops ultraviolet-induced erythema, a prostaglandin (PG)-mediated function. When PGE_2 (100 μg) was injected into the white ring, redness appeared, indicating that the whitened area was not due to vessel unresponsiveness and suggesting a decrease in endogenous PGE_2 levels.

Inhibitors of PG synthesis were detected in an assay system by measuring their effect on the oxidation of arachidonic acid (AA) by sheep vesicular gland PG synthetase. Aliquots of whole-skin homogenate from normal control patients (10), uninvolved skin from patients with psoriasis (11), psoriatic plaque (13), and the white ring (3) were incubated with AA and PG synthetase to determine the levels of oxygen consumption relative to the control (PG synthetase and AA alone). Extracts from normal control skin and uninvolved psoriatic skin consumed +155 and +260 μm O_2/min/mg protein, respectively, whereas extracts from psoriatic plaque and the white ring had significantly lower values at −20 and −200.

PGE_2 levels determined by gas chromatography in the uninvolved psoriatic skin (7), normal control skin (6), plaque (6), and white ring (3) were 350, 520, 394, and 121 ng/mg, respectively—signifcantly decreased in the ring.

These results indicate the presence of an inhibitor or inhibitors of PG synthesis in the psoriatic plaque which, during ultraviolet-light and coal-tar therapy, diffuse outward to produce the white ring of Woronoff.